Dendritic Cells

Toebak, M.J.

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Summary and discussion

Allergic contact dermatitis (ACD) results from a T-cell-mediated, delayed-type hypersensitivity immune response induced by allergens. Following encounter with an allergen, dendritic cells (DCs) become activated and undergo maturation and differentiate into potent immunostimulatory DC, and are able to present antigen effectively to T-cells[1]. The epidermal DCs, Langerhans cells (LCs) are believed to serve as sentinels of the immune system, recognizing and responding to changes in the microenvironment and encounter with foreign antigens. In contrast to ACD, irritant contact dermatitis (ICD) is described as a non-immunologic local inflammatory reaction to a broad range of chemicals with no or little T-cell stimulating properties. Histopathology of allergic and irritant reactions can be remarkably hard to distinguish. The similarity may be explained by the fact that the early inflammatory reaction of ACD is non-specific of nature and is induced by the irritative capacity of an allergen[2]. Furthermore, most allergens can act as irritants at a high enough dose.

The immunological mechanisms underlying ACD and ICD reactions are different. However, both phenomena result in vasodilatation, up regulation of endothelial adhesion molecules, mast cell degranulation, keratinocyte cytokine and chemokine production, influx of leukocytes, and LC migration into the dermis. Differences between allergen and irritant exposure have been studied in murine models and humans. In mice, MHC class II, IL-1α, IL-1β, CXCL10 and CXCL2/CXCL3 were found to be only up regulated after allergen painting. In humans, our group showed that expression of CXCL9, CXCL10 and CXCL11 has been reported to be specifically up regulated in ACD and not in ICD[3]. Of course, true differences between an allergic and irritant reaction depend on whether or not allergen-specific T-cells become involved. Only those chemicals with sufficient affinity for MHC molecules, and for which matching T-cell receptors exist can ultimately cause ACD. As DC maturation is required for proper T-cell activation, one can speculate that allergens and not irritants are able to sufficiently activate maturation of skin DCs. Indeed, *in vitro* studies showed that potential contact allergens can be distinguished from irritants by their different ability to initiate LC/dermal DC migration and maturation[4;5].

Importantly, the frequency of allergic skin disorders has increased in the last decades.

Therefore, the identification of potential sensitizing chemicals has gained importance in the view of skin safety. Traditionally, predictive testing for allergenicity has been conducted in animal models. For ethical and regulatory reasons, animal use for sensitisation testing of compounds for non-medical use, e.g. for cosmetic purposes, will shortly be banned. Therefore, new non-animal based test methods need to be developed. Many studies have shown the potency of classification methods of contact allergens, however no solid *in vitro* studies for allergen detection have been developed as of yet and, therefore, the existing methods need refinement. The main objective of the research described in this thesis was to further explore the use of DCs in skin sensitization hazard identification.

In **chapter 2**, we investigated the role of skin residential cells (keratinocytes and fibroblasts) in the induction phase of chemical exposure. For this study, we made use of a human skin equivalent model (HSE), in which only skin keratinocytes and fibroblasts are present. Exposure of these HSEs with chemicals (either allergen or irritant) resulted in secretion of the pro-inflammatory cytokines, IL-1α and TNF-α. Subsequently, these cytokines stimulated the production of chemokines, notably CXCL8 and CCL20. Allergen- and irritant-mediated CCL27 secretion occurred in an IL-1α and TNF-α independent manner. In summary, we showed that there is no difference between allergens and irritants with regard to chemokine and cytokine production by keratinocytes and fibroblasts. Both allergens and irritants can induce secretion of general alarm signals by skin cells.

Chapter 3 described an *in vitro* assay for sensitisation testing based on DC responses. Our results showed that an increased production of the chemokine CXCL8 of monocyte-derived (mo)DCs could only be induced by allergen exposure. In contrast, irritant exposure of moDCs led to a decreased CXCL8 production. CXCL8 was the only chemokine amongst six chemokines tested whose secretion was found to significantly increased after exposure to all seven allergens tested. Thus, CXCL8 measurements in moDCs may provide a novel tool to identify contact allergens *in vitro*. The fact that CXCL8 secretion is only increased upon exposure to contact allergens and not contact irritants suggests a critical role of CXCL8 in allergic responses. However, the specific role of CXCL8 is still unknown.

In line with chapter 3, a new methodology, kinomic profiling to identify allergens and irritants was explored in **chapter 4**. Using MUTZ-3 progenitor cells as a model for DCs, we used kinomic profiling to evaluate signal transduction pathways involved in allergen-mediated DC maturation. We investigated a broad panel of kinases involved in e.g. membrane receptor, phospholipase, MAPK and cell cycle pathways in order to determine whether this approach may be suitable for distinguishing allergens from non-allergens. In this way, we identified potential target peptides, which are substrates for specific kinases in each of these pathways. The 74 selected peptides and their respective kinases now require a follow-up study involving extensive screening with a panel of test chemicals consisting of both sensitisers and non-sensitisers.

Chapter 5 described whether contact and respiratory allergens possessed intrinsic capacities to polarise DC towards secretion of type-1 or type-2 cytokines. Maturation of moDCs in the presence of the prototypic contact allergens DNCB and OXA induced strict type-1

DC polarisation. In contrast, the respiratory allergens TMA and Der p1 showed strict type-2 DC polarisation. The contact allergen $NiSO_4$ induced both type-1 and type-2 DC features. These results support the view that allergens have an intrinsic capacity to skew immune responses at the DC level, irrespective of local factors such as determined by cutaneous or mucosal epithelial microenvironments.

In **chapter 6**, we compared DC cytokine profiles of atopic individuals to healthy controls in response to allergen exposure. We showed that stimulation of moDCs from atopic individuals to protein allergens house dust mite and grass pollen resulted in a more pronounced type-2 cytokine release as compared to healthy controls. As to chemical allergens (DNCB, TMA and $NiSO_4$), no significant differences could be observed in cytokine balance of DCs between healthy and atopic individuals.

In **chapter 7**, the modulatory effects of anti-inflammatory drugs on DC maturation and polarisation have been studied. We demonstrated that all anti-inflammatory drugs tested, except for lactoferrin, had clear dose-dependent inhibitory effects on DC maturation, as shown by suppression of CD86 expression or CXCL8 secretion. The glucocorticosteroids hydrocortisone and dexamethasone suppressed exclusively release of type-1 cytokines. A less pronounced, but similar profile was observed for di-methyl-fumarate and 1-α,25-dihydroxyvitamin D_3. Cyclosporine A suppressed both type-1 and type-2 cytokine secretion. In contrast, diclofenac selectively suppressed type-2 DC cytokine secretion. In conclusion, anti-inflammatory drugs displayed differential suppressive effects on DC polarisation and maturation. The present results give more insight into the pharmacological effects of immunosuppressive drugs on the immune system, and can thereby contribute to a more rational selection of anti-inflammatory drugs in the treatment of inflammatory skin disorders.

DENDRITIC CELLS: TOOLS FOR ALLERGENS SCREENING

The research described in this thesis had the main aim to identify new *in vitro* markers, which can be used as a predictive assay for contact allergens. It is now well-established that DCs play a pivotal role in the orchestration of adaptive immune responses, and in the activation and regulation of T-cell functions. Therefore, most cell-based assays for risk assessment for chemicals are based on DCs. As example, we described in chapter 3 that CXCL8 secretion measurements can discriminate allergens from irritants by using moDCs. Our observation is confirmed on RNA levels; Szameit *et al.* (2008) showed that allergens but not irritants induce expression of CXCL8 mRNA[6]. A number of researchers have monitored the responses of moDCs to various allergens and reported alterations in e.g. receptor-mediated endocytosis[7], surface marker expression[8], gene marker expression[9], signal transduction[4;10]. Although these markers were reported not to be induced by irritants, their use for screening purposes still needs to be confirmed. Importantly, these data support the view of the use of moDCs and their endpoints as a potential *in vitro* approach for identification of allergens.

In addition, moDCs might also be used to predict the type (type-1 or type-2) as a response to allergen exposure as described in chapter 5. In general, we have demonstrated that DCs

secrete type-1 or type-2 cytokines in response to prototypical type-1 or type-2 allergen, respectively. These data indicate that DCs can not only be used to identify sensitising compounds but might also be used to determine the polarising potentials (type-1 or type-2) of contact allergens.

Human donor variability remains a real problem when studying the effect of contact allergens *in vitro*[11]. Atopy, the predisposition to develop immediate type hypersensitivity reactions, may contribute to variability seen in dendritic cell responses to chemical allergens. In chapter 6, we demonstrated that DCs from atopic individuals induce a stronger polarisation cytokine production profile when exposed to allergens like house dust mite as compared to healthy individuals. Such donor-to-donor variability should be taken into account during the development of a robust *in vitro* predictive test system for sensitisers.

Next to moDCs, DCs can also be generated from other sources: $CD34^+$-progenitor cells or DC cell lines. Generating DCs from $CD34^+$-hematopoietic cell precursors is relatively time-consuming and cell numbers are limited. Alternatively, there is great interest in the use of DC cell lines. The great advantage of using cell lines is the lack of donor variability and cell lines are easy to obtain and grow. Therefore, various cell lines have been explored as potential DC surrogates including THP-1[12], KG-1[13], U937[14], MUTZ-3[15]. For example, it was reported that THP-1 cell line up regulates CD54 and CD86 expression in response to allergen exposure[12]. Initial investigations indicated that the U937 cell line might be an alternative approach for allergen screening. In line with our results described in chapter 3, Python *et al.* (2007) showed that the U937 cell line induced CXCL8 gene expression following allergen treatment[14]. One of the major limitations to the above mentioned studies is the number of potential markers tested. Normally, with flow cytometry and ELISA only a handful of markers are tested. Nowadays, new technology such as micro-array or pepchip analysis, like discussed in chapter 4, might be used to determine an allergen-specific DC activation profile.

Only recently, the use of micro-arrays for assessment of a predictive test for contact allergens was introduced[6;16;17]. Szameit *et al.* (2008) showed that micro-array analysis of allergen-treated DC revealed clear differences of gene expression patterns between cells treated with allergens ($NiSO_4$ and Brandrowski's Base) and cells treated with an irritant (SDS)[6]. A targeted micro-array containing oligonucleotide probes for 66 immune-relevant genes was developed. The genes upregulated after 24 hours allergen exposure were CXCL8, CCL17, CCR7, CCL22, CD86, CXCR4, TNFRSF1A (TNF receptor superfamily member 1A). The micro-array data could be confirmed by PCR for all genes, except TNFRSF1A. Importantly, all markers that were identified in this study are also known to be involved in DC maturation and migration. The results of this study seem to be very useful to elicit changes in expression of immune-relevant genes after treatment of chemical allergens and irritants. These observations warrant further large scale analysis panels of a broader panel of potential contact allergens (weak, moderate and strong) and irritants.

As earlier stated, skin DCs have the role to translate antigenic signals at skin surface into adaptive immune response. In ACD, an allergen is captured by LCs or dermal DCs for further processing which will lead to an immune response in an allergic individual. To do so, DCs are helped by locally available cytokines and chemokines. In particular keratinocytes,

as the main cell population in the skin, may provide additional cytokines and chemokines that orchestrate the movement and function of skin DCs. As we described in chapter 2, skin cells secrete alarm signals which consist of primary and secondary signals. We showed that IL-1α and TNF-α are released as primary alarm signals by keratinocytes and fibroblasts, which trigger the release of secondary chemokine (e.g. CCL20, CXCL8) alarm signals. This observation is supported by *in vivo* and *in vitro* studies in which both allergen and irritant exposure results in increased cytokine levels in keratinocytes and fibroblasts[18-19].

It is known that some allergens need metabolic activation within the skin. Skin-sensitising chemicals are either naturally protein active or have to be metabolised to a protein-reactive compound (i.e. pro-haptens). It is generally believed that approximately 20% of skin sensitising chemicals can be described as pro-haptens[20]. Current DC-based allergen identification tests are probably unable to identify such pro-haptens. Therefore, in order to identify all contact allergens a test model resembling as closely as possible the human situation might be needed. The need for signals additional to that provided by the allergen itself asks for the requirement for the presence of additional skin cell types within the culture system. For example, we demonstrated that keratinocytes and fibroblasts secrete the cytokines TNF-α and IL-1α (chapter 2). In particular TNF-α is known to be able to enhance DC responses[21]. Therefore, one might speculate that a culture system in which all three cell types (KCs, fibroblasts and DCs) are combined could be a sensitive *in vitro* assay for allergen identification (e.g. weak allergens or pro-haptens). This approach is currently being further explored.

PERSPECTIVES

Risk assessments of new industrial chemicals and cosmetic ingredients serve to ensure that skin exposure to skin sensitisers is carefully managed in accordance with their sensitising potential. Over the past years, much effort has been made to refine *in silico, in vitro* and *in vivo* methods which are capable of identifying sensitising compounds. In the last years, the murine local lymph node assay (LLNA) has become the preferred test method for assessing skin sensitisation potential. From ethical and legislative perspective, animal use for sensitisation testing of compounds for non-medical use, e.g. for cosmetic purposes, will be banned. Therefore, new non-animal based test methods need to be developed and the search for alternatives to animal for the identification of sensitisers has already been started. New approaches to developing information for risk assessment based on computational (quantitative) structure-activity relationship [(Q)SAR] methods may be complementary to and reduce the need for animal testing and *in vitro* testing. SAR models rely on the use of database containing physical-chemical properties of experimentally examined compounds and evaluate their relationship to bioactivity. Unfortunately, the current status of the QSAR approach is considered initial.

In this thesis, the use of DC-based models for assessment of the sensitising potential

of a compound is described. DC can be exploited for identification of chemical allergens but also to determine the type of immune response. In future, the most robust method to identify the sensitising capacity of allergens might benefit from a broad panel of markers. Available markers from e.g. genomics, kinomics, proteomics should be combined to one *in vitro* DC assay for allergen screening. At last, the final goal should be to develop a non-animal, simple, accurate and cost-effective assessment of the sensitising potential of a compound. Successful development, acceptance and implementation of such a model are highly dependent on collaboration between research, industry and government.

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